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Uptake of Triticonazole, during Imbibition, by Wheat Caryopses after Seed Treatment

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Abstract: The uptake of ^{14}C-labelled triticonazole by wheat caryopses during imbibition was investigated. The uptake from an aqueous solution appeared to be driven by mass flow rather than by accumulation in seed lipids. During treatment with a liquid seed-dressing preparation of triticonazole, *c.* 1 μg triticonazole per caryopsis (2·4% of applied triticonazole) entered the seed. During germination in soil, another *c.* 1 μg triticonazole per caryopsis entered the seed in 24 h. In killed seeds, no penetration was observed between 24 h and 72 h after the beginning of imbibition. After seed treatment and imbibition in soil, triticonazole appeared to be located in the seed coats and embryo, but not in endosperm; experiments suggested that the testa acted as a barrier. Under our conditions, the pathway from seed coats to shoots was not an important route for triticonazole uptake by the shoots.

Key words: triazole, *Triticum aestivum*, seed treatment, imbibition, uptake

1 INTRODUCTION

Systemic pesticides have been used for cereal seed treatment since the 1960s with the 1,4-oxathiin fungicides[1] against loose smut (*Ustilago nuda* and *Ustilago tritici* (Jens.) Rostr.) and later, ethirimol[2] and triadimenol[3] against powdery mildew (*Erysiphe graminis* DC). New prospects appeared with the advent of two broad-spectrum active ingredients: the fungicide triticonazole[4] and the insecticide imidacloprid.[5] Some data are available on the routes of uptake of systemic pesticides used as seed treatment, but the treatments were performed with diverse modes of application. The mechanisms of pre-emergence herbicide uptake in soybean seed were studied by soaking seeds in aqueous solutions of herbicides.[6–9] The absorption pattern was found to depend on the physicochemical properties of the active ingredient. Lipophilic compounds are taken up in soybean seed via diffusion,[6,8,9] and are accumulated in seed tissues by trapping processes, probably into lipids.[10,11] More polar compounds are less accumulated,[6,8,9] and their uptake may depend more on mass flow.[9]

The translocation of various pesticides from seed to shoots was demonstrated using seeds soaked in aqueous solutions or suspensions of pesticides. Translocation was effective in rice (*Oryza sativa* L.) seedlings for carbendazim[12] and pefurazoate[13] and in cowpea (*Vigna unguiculata* (L.) Walp.) for metalaxyl.[14] In the case of seed dressing, the active ingredient is deposited onto the seed surface and may be released to the soil and then be absorbed by roots. As far as cereals are concerned, from the two- to three-leaf stage to the heading phase, systemic pesticides are taken up by roots and translocated to the shoots.[15–18] The site of entry from sowing to the two- to three-leaf stage is still under discussion. The micropyle may be a route of entry but to our knowledge this pathway has not been studied. According to Graham-Bryce *et al.*,[19] the uptake of systemic pesticides during this period depends on their availability in soil solution, which is related to their physicochemical properties. On the other hand, Thielert *et al.*[20] claimed that the main pathway of triadimenol uptake by shoots until the two- to three-leaf stage was the pathway from seed coats to endosperm to scutellum to shoots.

The aim of our study was to examine the seed uptake of triticonazole during imbibition of seed-treated wheat

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caryopses and to shed some light on the involvement of the imbibition phase in the uptake of active ingredient by the shoots.

2 MATERIALS AND METHODS

2.1 Uptake of ^{14}C-labelled triticonazole from an aqueous solution by untreated seeds

Spring wheat seeds (*Triticum aestivum* L., cv. Rex) were allowed to imbibe using aqueous solutions of [*phenyl*-U-^{14}C] triticonazole (specific activity = 3·71 kBq μg^{-1}). [^{14}C]Triticonazole (50 μg) was dissolved together with unlabelled triticonazole (125 μg) in acetone (1 ml). The solvent was then evaporated and the active ingredient was redissolved in water (25 ml) to give a final concentration of 7 mg litre^{-1}. After weighing, wheat seeds were placed in Petri dishes (7 cm diameter, 20 caryopses per dish) between two layers of germination paper (Germaflor 160 g). Triticonazole solution (5 ml per dish) was then poured onto the paper. After 1, 3, 6, 12 and 24 h, the seeds were removed, rinsed three times with distilled water (2 ml per seed), dried with filter paper and weighed. The seeds were then combusted in a Packard 306 Oxidizer and the radioactivity was measured by liquid scintillation counting (LSC) in a Beckman LS 6000 TA counter. Radioactivity in the imbibition solutions was measured by LSC at the beginning and at the end of each experiment (10-μl aliquots). For each experiment, 20 replications of one caryopsis were performed.

2.2 Uptake of [^{14}C]triticonazole from treated seeds

2.2.1 Seed treatment

Spring wheat seeds were treated using a liquid seed-dressing formulation of [^{14}C]triticonazole ('Real' 200 g litre^{-1} SC, Rhône-Poulenc Agro). The seeds (70 g) were treated with triticonazole (140 mg) corresponding to the required dose rate of 2 g AI kg^{-1}. The treatment slurry was prepared by mixing suspension concentrate (0·7 ml) with water (0·7 ml). The treatment was performed by mixing the seeds with the liquid slurry for 1 min and the seeds were then allowed to dry for 1 h at room temperature. [^{14}C]Triticonazole was 3·2% of the total active ingredient. To check the treatment efficacy, 50 treated caryopses were combusted and the radioactivity was measured by LSC. This allowed the calculation of the mean applied radioactivity, the active ingredient equivalent per seed and the dose rate (g kg^{-1}). The amount of triticonazole applied with 95% confidence interval was 81·2($\pm$4·6) μg per caryopsis which is equivalent to 1·8($\pm$0·1) g kg^{-1} seed.

2.2.2 Imbibition

The imbibition was performed in a growth chamber in plastic pots (2·2 cm diameter, 6 cm height, 1 caryopsis per pot) filled with a mixture of silt loam soil + sand (1 + 1 by volume, 8 g per pot), at 80% RH and 18°C in darkness. After weighing, one treated seed was sown in each pot at a 2-cm depth. Distilled water (4 ml per pot) was added. Uptake of triticonazole by wheat caryopses was determined in dry treated seeds, and after seeds were allowed to imbibe for 24 and 72 h. The radioactivity was measured in soil, in percolate, in seed dressing and in caryopses. For the 72-h experiments, killed seeds were used to prevent seed germination. Two methods were used to kill the seeds: (1) treated seeds (1 g) were heated at 80°C for 48 h, (2) treated seeds (1 g) were exposed to a Cobalt 60 source at a 20-kGy dose. For both treatments, no germination occurred after 72-h imbibition and the seeds were assumed to be dead. For each experiment, 20 replications of one caryopsis were performed.

2.2.3 Penetration of triticonazole into the seeds

Each caryopsis was washed with water (2 ml per caryopsis), dried with filter paper and weighed. Absorption was evaluated by washing the caryopses with acetonitrile (5 × 10 s, 2 ml per caryopsis) and then wiping it four times with filter paper. To verify washing efficacy, each caryopsis was briefly sonicated in acetonitrile (3–4 s, 2 ml per caryopsis). All the seeds whose recovery in the last washing was higher than 100 ng triticonazole per caryopsis were excluded from the analysis. The radioactivity in washes and in filters was considered not to have penetrated. The seeds were then combusted and the penetrated radioactivity was measured by LSC. The soil samples were air-dried and extracted for 1 h with methanol + water + ammonia (7 + 2 + 1 by volume, 2 ml g^{-1} dried soil). The extraction mixtures were filtered. The volume of the filtrates was measured and aliquots (1 ml) were used for LSC. For each experiment, 20 replications of one caryopsis were performed.

2.3 Localization of [^{14}C]triticonazole in wheat caryopses

Wheat seeds treated with [^{14}C]triticonazole as described in Section 2.2.1 were allowed to imbibe for 24 h in the soil–sand mixture. The seeds were then washed by the technique described in Section 2.2.3 and placed in a glutaraldehyde solution (25 g litre^{-1} in phosphate buffer, pH 7·0) overnight at 4°C. The seeds were then removed and rinsed (5 × 30 s) with phosphate buffer (pH 7·0). Longitudinal sections (100 μm thickness) were performed in phosphate buffer by means of a Bio Rad Micro-Cut H200 vibratome. The sections were placed on polylysine-coated plates and dried at 40°C for 30 min. The plates were exposed to an auto-

radiography film (Amersham β-max). After a three-day exposure at room temperature, development was followed by observation with a photonic microscope. To check whether glutaraldehyde fixation had induced artifacts, the same procedure was carried out without fixation.

Untreated wheat seeds were allowed to imbibe for 24 h on germination paper (Germaflor 160 g). Longitudinal sections were performed as described above, and the sections were incubated in a solution of [^{14}C]triticonazole at a concentration of 7 mg litre^{-1} (7·4 MBq litre^{-1}). After 1 h, the sections were removed, rinsed with distilled water (3 × 5 s) and chilled with liquid nitrogen. The sections were then exposed to an autoradiography film (Amersham β-max). After a five-day exposure at −20°C, development was followed by observation with a photonic microscope.

2.4 Behaviour of triticonazole taken up during imbibition

Wheat seeds (30) treated with [^{14}C]triticonazole were allowed to imbibe for 24 h as described in Section 2.2.3. The caryopses were then recovered and divided into two batches. The first batch (10 caryopses) was directly sown in a plastic pot (20 cm diameter, 16 cm height) filled with a mixture of silt loam soil + sand (1 + 1 by volume). In the second batch, the caryopses (20) were washed by the technique described in Section 2.2.3 and then sown under the same conditions (10 seeds per pot). Water was added (300 ml per pot) and the pots were placed in a growth chamber at 70/80% RH, 18/10°C, 16 h/8 h, light/dark. After 15 days, the plants were at the two-leaf stage and were sampled. Seeds, roots and shoots samples were combusted and the radioactivity was measured by LSC. The soil was air-dried and extracted three times with methanol + water + ammonia (7 + 2 + 1 by volume, 2 ml g^{-1} dried soil). The sample was filtered and the filtrate evaporated. Residues were redissolved with methanol (5 ml). Aliquots (1 ml) were used for LSC. The samples from the two pots were combined before analysis.

All the results are given as arithmetic means with 95% confidence intervals (CI).

3 RESULTS

3.1 Uptake of [^{14}C]triticonazole from an aqueous solution by untreated seeds

After imbibition on filter paper, the concentration of the triticonazole solution was 5 mg litre^{-1} and it exhibited no significant variation over the duration of the experiment (data not shown). As shown in Fig. 1, the initial rate of water uptake was high and decreased gradually. The rate of triticonazole uptake by the seed had a similar pattern. The amount of triticonazole absorbed reached 200 ng per caryopsis after 24 h. Triticonazole concentration in the water absorbed by the seed was calculated (C_{seed}). It was compared to triticonazole concentration in the external medium (C_{ext}). The accumulation ratio C_{seed}/C_{ext} showed little variation over 24 h and remained close to 2 : 1.

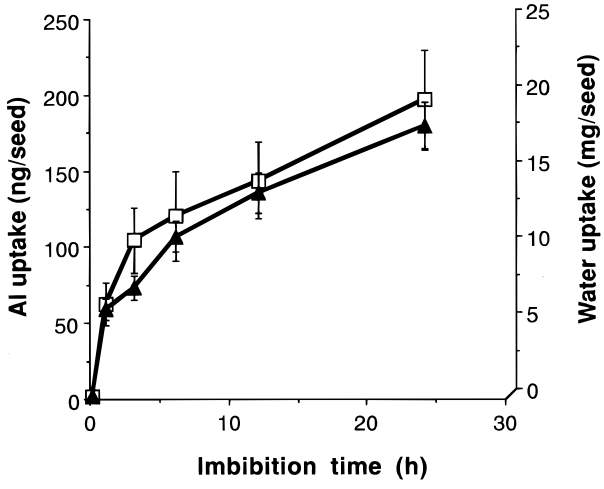

Fig. 1. Uptake of (□) triticonazole and (▲) water by untreated wheat seeds from a triticonazole solution (5 mg litre^{-1}). Mean values of 20 replicates, vertical bars represent 95% CI.

3.2 Uptake of [^{14}C]triticonazole from treated seeds

The amount of water taken up by the seed batches having undergone different treatments is shown in Table 1. No significant differences were found between the dry weights of the three batches used. From the data of Table 1 we calculated the amount of water absorbed by the seeds as expressed in % dry weight (Fig. 2). After 24 h imbibition, water uptake was similar in the living and in the radiation-killed seeds, but it is significantly higher in the heat-killed seeds (Fig. 2). After 72 h imbibition, water uptake was further increased to 54% and 72% for radiation-killed seeds and heat-killed seeds, respectively.

Distribution of triticonazole is shown in Table 2. A significant proportion of triticonazole moved from the seed dressing into the soil during the first 24 h, from living and killed seeds. This proportion did not increase from 24 to 72 h. Recovery of applied triticonazole ranged from 80 to 100%. Differences in recovery may be due to the variability of seed treatment. No triticonazole was detected in the percolates.

As shown in Fig. 3 for the living seeds, more than 1 μg triticonazole per caryopsis penetrated into the caryopses before imbibition. After 24 h imbibition, the amount of triticonazole absorbed reached 2 μg per

TABLE 1
Amount of Water taken up after Imbibition, by Living Seeds, Heat-Killed Seeds and Radiation-Killed Seeds.[a]

	24 h		*72 h*	
	Dry weight ($mg\ seed^{-1}$)	*Water uptake* ($\mu l\ seed^{-1}$)	*Dry weight* ($mg\ seed^{-1}$)	*Water uptake* ($\mu l\ seed^{-1}$)
Living seed	45·7 (±3·3)	16·2 (±1·2)	—	—
Heat-killed seed	41 (±2)	22·2 (±1·5)	37·6 (±3·3)	27·4 (±3·4)
Radiation-killed seed	37·4 (±4·6)	13·6 (±1·7)	40·4 (±4·3)	21·7 (±2·4)

[a] Mean values of 20 replicates with 95% CI.

caryopsis. The amount of triticonazole absorbed by living or radiation-killed seeds was not significantly different after 24 h. For the heat-killed seeds, the amount penetrated was two to three times higher. The amount of triticonazole absorbed by killed seeds did not increase from 24 to 72 h. The penetration in heat-killed seeds remained three times higher than in radiation-killed seeds.

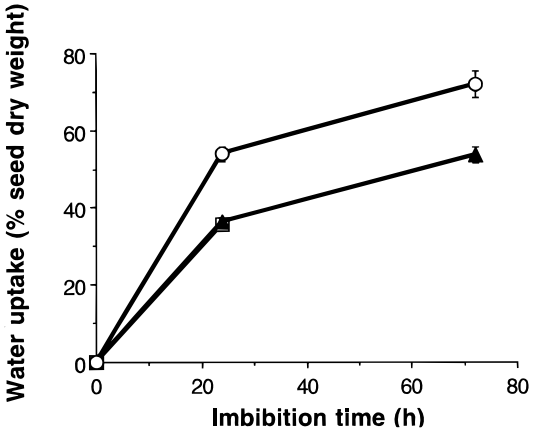

Fig. 2. Water uptake by (□) living seeds, (○) heat-killed seeds and (▲) radiation-killed seeds after 24 and 72 h imbibition in soil. Mean values of 20 replicates, vertical bars represent 95% CI.

3.3 Localization of [^{14}C]triticonazole in wheat caryopses

As shown in Fig. 4(a), [^{14}C]triticonazole was present almost exclusively in the embryo and seed coats after seed treatment and 24 h imbibition. Similar results were obtained without glutaraldehyde fixation (data not

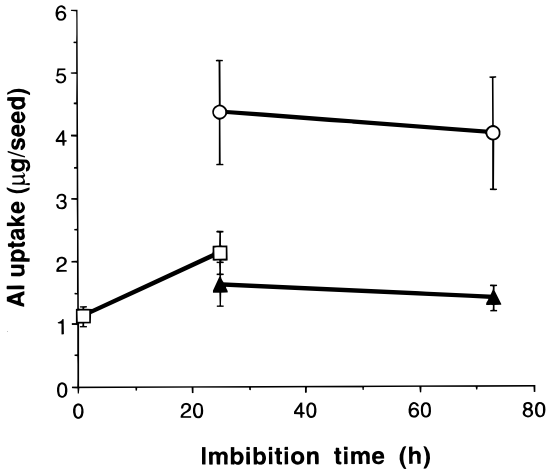

Fig. 3. Uptake of AI by [^{14}C]triticonazole-treated seeds after 24 and 72 h imbibition in soil. The seeds were (□) living, (○) heat-killed or (▲) radiation-killed. Mean values of 20 replicates, vertical bars represent 95% CI.

TABLE 2
Distribution of Triticonazole in Soil and Seeds (% Applied Dose) after Imbibition.

	Triticonazole (% of applied dose)[a] (95% CI)		
	Soil	*Seeds*	*Recovery*
24 h imbibition			
Living seeds	24·2 (±3·9)	77·6 (±9·0)	101·8 (±8·6)
Heat-killed seeds	25·2 (±4·2)	68·6 (±5·7)	93·8 (±6·6)
Radiation-killed seeds	17 (±2·3)	70·4 (±8·7)	87·9 (±9·4)
72 h imbibition			
Heat-killed seeds	24·0 (±2·3)	56·7 (±10·5)	80·9 (±11·4)
Radiation-killed seeds	23·3 (±2·3)	74·0 (±9·0)	97·5 (±10·2)

[a] Mean value of 20 replicates.

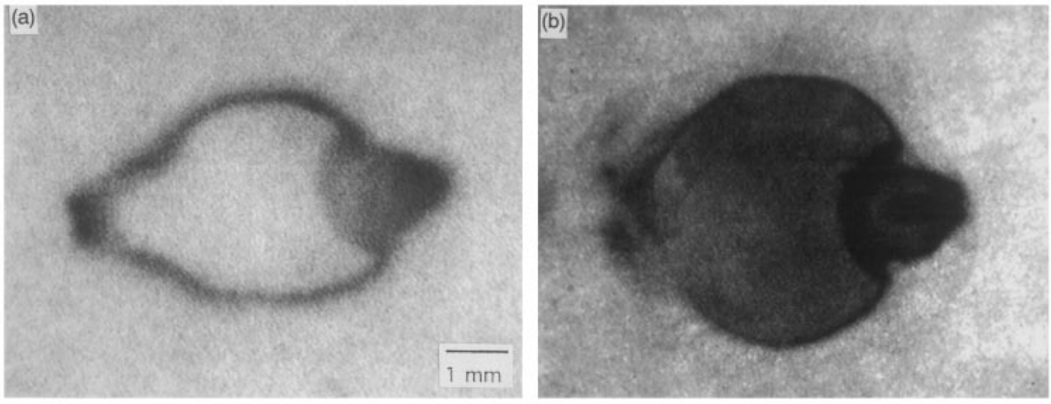

Fig. 4. (a). Autoradiogram of a longitudinal cut of a [^{14}C]triticonazole-treated wheat caryopsis imbibed for 24 h in soil. (Enlargement × 12). (b) Autoradiogram of a longitudinal cut of a 24 h imbibed untreated wheat caryopsis after 1 h incubation in a [^{14}C]triticonazole solution. (Enlargement × 12).

shown). Figure 4(b) shows that when untreated caryopses were longitudinally sectioned and incubated in a [^{14}C]triticonazole solution, the label was found in all seed parts, even in the endosperm. However, some accumulation was observed in the embryo and in the seed coats.

3.4 Behaviour of triticonazole taken up during imbibition

When treated seeds were allowed to imbibe for 24 h then washed and sown in pots to be grown until the two-leaf stage, 66% of triticonazole absorbed by the seeds during imbibition was found in the soil at the two-leaf stage (data not shown). About 23(±5)% remained in the seed, but only 8(±1·5)% was recovered in the shoots. As shown in Fig. 5, when the caryopses were not washed after imbibition, the amount of triticonazole taken up in wheat shoots at the two-leaf stage was five to six times higher than with washed caryopses. Root uptake of triticonazole at this stage was 15 times higher when the seeds were not washed after imbibition.

4 DISCUSSION

The uptake of triticonazole by wheat seeds from an aqueous solution had a different pattern to that previously described for different herbicides and soybean seeds.[6–9] For linuron Rieder *et al.*[6] found in soybean seeds a 28 : 1 accumulation ratio from a 20 mg litre^{-1} herbicide solution, whereas for triticonazole the accumulation ratio reached only 2 : 1 after 24 h imbibition. The accumulation of linuron resulted from trapping processes, presumably into soybean seed lipids since linuron is lipophilic: its log K_{ow} (octanol-water partition coefficient) is 3·0 and its water solubility is 81 mg litre^{-1}.[21] Triticonazole is also lipophilic (log K_{ow} = 3·3, water solubility = 7 mg litre^{-1})[22] but the lipid content of wheat seeds is low (2–3% versus 15–25% for soybean seeds[8]). Hence, triticonazole uptake by mass flow may

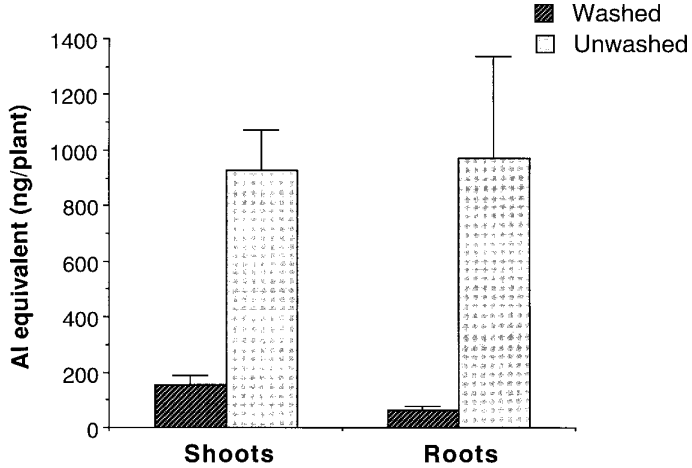

Fig. 5. Uptake of triticonazole in wheat shoots and roots at the two-leaf stage, 15 days after sowing. The seeds were treated with [^{14}C]triticonazole, imbibed 24 h in soil and were washed or not after imbibition. Mean values of 20 replicates for washed seeds and 10 replicates for unwashed seeds, vertical bars represent 95% CI.

be a more important process than accumulation into lipids.

The amount of triticonazole taken up by dressed caryopses after 24 h imbibition (2 μg per caryopsis) was twice the amount of triticonazole found in the shoots 15 days after sowing. Triticonazole detected in the seeds at the end of imbibition had penetrated during two distinct periods of time. Firstly, to prepare triticonazole-dressed seeds the latter were treated with a liquid slurry containing the fungicide. Uptake (*c.* 1 μg per caryopsis) probably took place as long as the dressing had not dried. Secondly, triticonazole penetrated during the imbibition experiment (*c.* 1 μg per caryopsis). To our knowledge, no study on the penetration of active ingredient into seeds following seed dressing with an aqueous suspension concentrate (SC) has been yet published. We show here that it can be as important as penetration during imbibition of the seeds. The amount of triticonazole taken up during seed imbibition was five times higher than the amount absorbed from an aqueous triticonazole solution at a concentration close to its water solubility. This increase was probably due to the presence of adjuvants in triticonazole seed-dressing formulation.

The use of killed seeds showed that no further triticonazole penetration occurred from 24 to 72 h, although water uptake continued. The uptake of water and triticonazole by heat-killed seeds was significantly higher than that observed on living or radiation-killed seeds. Killing seeds by heat-treatment probably induced an artifact (mechanical damage to the seed coats or alteration of its structure can be expected). The results obtained with this technique were discounted.

Triticonazole could be accumulated in the endosperm provided that this tissue was made accessible to it. The absence of triticonazole in endosperm after imbibition of treated wheat seeds may be due to impermeability of the testa and may not result only from partitioning of triticonazole into seed lipids. Similar results were reported on wheat seeds treated with [^{14}C] triadimenol.[16] Conversely, benomyl was shown to accumulate in wheat endosperm after seed treatment.[23] This discrepancy with our results is difficult to interpret since, contrary to triticonazole, benomyl is a hydrophilic compound and since benomyl treatment was performed with a dry powder.

Although the amount of triticonazole absorbed at the send of imbibition was important, it was not involved to a great extent in the uptake of triticonazole by the shoots at the two-leaf stage. Moreover, one must take into account that in our experimental design, desorption of triticonazole from washed seeds into soil, then uptake by the roots and subsequent translocation to the shoots was likely to occur. Additionally, the amount of triticonazole taken up by the shoots may originate partly from direct uptake by the embryo during imbibition. Hence, the pathway from seed coats to endosperm to scutellum to shoots does not seem to be an important route of uptake by the shoots, possibly because the barrier function of the testa may persist for a long time. Similar results were found with wheat caryopses which were seed-dressed with triadimenol.[16] The testa remained a barrier for triadimenol for at least six days after sowing. On the other hand, in another study, the pathway from seed coats to endosperm to scutellum to shoots was claimed to be the main way of triadimenol uptake by the shoots until the two-to three-leaf stage.[20] Translocation of carbendazim[12] and pefurazoate[13] from seed to shoots was observed in rice. In the latter studies, the seed treatment was made by soaking the seeds for 24 or 72 h in an aqueous suspension of active ingredient. The fact that the species and the seed-treatment technique were different from ours prevents us from drawing conclusions.

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